

# Prevalence of *Ureaplasma urealyticum* in Endocervical Specimens of Female Patients in Qazvin, Iran

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## Abstract

**Background:** *Ureaplasma urealyticum* that is the smallest free-living bacterium does not have bacterial cell wall. These organisms cause different infections in respiratory and urinary tract system in close contact with epithelial cells. The current study aimed to determine the prevalence of *U. urealyticum* in endocervical samples of female patients referred to Kowsar hospital in Qazvin, Iran.

**Methods:** The study was conducted on 232 married females aged 20 - 50 years. According to a gynecologist's request, genital tract biopsies were taken from each individual using Dacron swabs. Each swab was placed into 4 mL of the pleural pneumonia-like organism (PPLO) broth media. Then 25  $\mu$ L of the suspension was inoculated on the surface of PPLO agar. The remaining broth media and agar plates were incubated at 35°C in 5% CO<sub>2</sub> atmosphere.

**Results:** Out of 232 tested samples, 87 cases (37.5%) were positive for *U. urealyticum* and 145 (62.5%) were negative.

**Conclusions:** Rapid laboratory detection of genital *Ureaplasma* in pregnant females is very important, mainly because of the ability of the bacteria to colonize the endocervical lining and cause injury to the fetus

**Keywords:** *Ureaplasma urealyticum*, Endocervical, Prevalence

## 1. Background

*Ureaplasma urealyticum*, the smallest free-living bacteria (1), lacks a cell wall and its cell membrane contains sterols and these features are unique among bacteria (1, 2). *Ureaplasma* species primarily colonize the mucus of the respiratory system and the urogenital tract and have a close relationship with the epithelial cell of their hosts. These organisms adhere to their own surface organelles to the mucosal surfaces of the genital tract and are founded in the cervical mucus and vaginal surfaces of 40% - 80% of adult females without clinical symptoms (2-4) and there is a common symbiotic relationship between these organisms and urinary tract organs in healthy people (4-7). According to the studies, the prevalence of this microorganism in Iran is reported 30% - 40% (8). These organisms cause different infections in the respiratory system and urinary tract in close contact with epithelial cells. Colonization of microorganisms in genitourinary tract are more common in young people, the ones with low socioeconomic status, sexual activity, multiple sexual partners

(3, 4), concurrent bacterial or protozoan infections (4) and also the black race (3).

The accession of infection is related to using oral and vaginal contraceptives, menstrual cycle and pregnancy (3, 4). *Ureaplasma urealyticum* is known as one of the non-gonococcal urethritis factors (NGU) (1, 2, 5, 7, 9), acute prostatitis and acquired arthritis in males. Also these bacteria are known as the main cause of infertility, miscarriage, sudden abortion, stillbirths, preterm births, chorioamnionitis (2, 3, 5) and postpartum endometritis in females.

Pneumonia, bacteremia and meningitis (2, 5) can be mentioned as related diseases in children and neonatal. According to aggravating condition of pregnant females, fetuses and neonates it is essential to use antibiotics for treatment. Tetracycline and quinolones are the drugs that can be used as a treatment for these bacteria (4, 7) and they have a moderate sensitivity to macrolides such as erythromycin, clarithromycin and roxithromycin (4). For pregnant females, children and infants, tetracycline should not be used and erythromycin is recommended instead. In the cases that tetracycline and erythromycin are

not effective, clindamycin, fluoroquinolones and others macrolides should be utilized (10). The criteria to identify *Ureaplasma* spp. in the genus level is based on the colony form and production of urease. The culture is a golden standard method to detect *Ureaplasma* spp. (2, 11) and production of urease discussed as a potential virulence factor (2). Colonies of *Ureaplasma* spp. are detectable by a diameter of 10 to 50 micrometers and have dark or brown appearance after 48 - 72 hours incubation at 37°C and CO<sub>2</sub> 5% (2, 3, 9). Other methods to diagnose this bacteria are molecular methods such as polymerase chain reaction (PCR) (2, 11). The problems of culture process which reduce the sensitivity of this method are a sensitivity of organism to drought and environmental conditions (2, 3), the osmotic changes and toxic metabolites (3). Considering the fact that the culture is considered as a standard, valuable and inexpensive method, designing modified methods to repair errors in previous experiments is essential.

## 2. Objectives

In the current study the *U. urealyticum* species were grown with changes in the cultivation process. Also the frequencies of *U. urealyticum* species were investigated through clinical specimens and their relationship with clinical symptoms in patients.

## 3. Methods

The current study was conducted on 232 female patients referred to the Kowsar hospital affiliated to Qazvin University of Medical Sciences in 2012. The population included all the married females patients aged 20 - 50 years. According to the diagnosis of a gynecologist, the subjects were selected for biopsy after completing the questionnaire and signing the written consent.

Inclusion criteria were as follows:

1. Having vaginal discharge, burning or itching in the vaginal area;
2. History of infertility;
3. History of miscarriage;
4. History of premature delivery;

Exclusion criteria were as follows:

1. Antibiotic consumption within a month before referring;
2. Being pregnant;
3. Passing menstrual period;

### 3.1. Sampling and Sample Transfer

The genital tract biopsies were performed after fitting disposable and sterile speculum and clearing secretions

by genital swab was conducted under the supervision of a gynecologist. Two samples were taken from each individual using Dacron swabs (polystyrene). The first swab, under sterile conditions, was placed into 4 mL of the pleural pneumonia-like organism (PPLO) broth. Each swab was entered into the endocervix for 1 cm, by applying a little pressure on the cervical mucus wall; it was rotated three times and taken out without contact with the other points on the way out. For further culture actions the first swab, under sterile conditions, will be put in 4 mL of PPLO broth. After moving several times within the media, it was immediately stored at 2 - 8°C and cultivated up to 24 hours.

The second swab was used to prepare two smear glass slides and the other one, as a store slide, was kept at -20°C.

#### 3.1.1. PPLO Broth

The broth media was consisted of culture media powder (Merck, Germany), 10% yeast extract, 20% de-complemented horse serum, 10% urea, 10% sucrose, 0.25% penicillin G, 0.1% amphotericin B and 0.2% phenol red.

#### 3.1.2. PPLO Agar

It was consisted of culture powder manufactured by Merck (Germany), 10% yeast extract, 20% de-complemented horse serum, 10% urea, 10% sucrose, 0.25% penicillin G and 0.1% amphotericin B.

#### 3.1.3. Cultivation and Transition From Broth to Solid Medium

Provided samples inoculated into PPLO broth were held for 24 hours in the refrigerator. Then for transition to solid culture under sterile conditions, the swabs were discarded after several moving in the PPLO broth. It was done to release mucus secretion and epithelial cells attached to the swabs into the broth. Then the broth was centrifuged under aseptically conditions for 15 minutes at 2,000 rpm. The supernatant was discarded under sterile conditions; 0.5 mL broth was added to uniform and dilute possible antibacterial compounds into sediment tubes. After uniform mixing of the broth medium, 25 µL of the suspension was inoculated by a loop on the surface of PPLO agar and cultivated as points, without spreading on the surface.

#### 3.2. Incubation

The remaining sedimentary and agar plates were incubated in the incubator (Memert, Germany) at 35°C in 5% CO<sub>2</sub> atmosphere.

The broth tubes were checked for change of color from yellow to purple and the plates were evaluated by an optical microscope (40X) for the presence of berries form brown or dark colonies. Under the condition of observing the color change in the broth medium and lack of

colonies in agar plates, based on the explained method, the broth cultures were transferred onto agar plates and the plates were checked again, respectively. The agar plates were daily checked to observe colonies up to 72 hours.

All samples with berry colonies (Figure 1) and changing color (Figure 2) were considered positive.



Figure 1. Berry Form Colonies of *Ureaplasma urealyticum* (40X)



Figure 2. Changing the Broth Medium Color is Observed in the Right Tube

Samples with changing of color in the broth medium without observing berry form colonies were considered negative. In microscopic examination, observing berry

form colonies of brown or black color (Figure 1) and confirmation of absence of other bacteria together with the color change of broth medium (Figure 2) was considered as a positive *U. urealyticum* culture.

#### 4. Results

Out of 232 samples in the current study, 87 (37.5%) cases were positive for *U. urealyticum*, and 145 (62.5%) were negative. According to the diagnosis of a gynecologist, 18 (7.8%) cases had a history of infertility, 56 (24.1%) with a history of abortion, 130 (56%) had signs of irritation or vaginal discharge and 13 (5.6%) had a history of the preterm labor. In the current study, out of 18 patients with a history of infertility, considering *U. urealyticum* culture, 7 (38.9%) were positive; 23 (41.1%) out of 56 patients with a history of abortion, 7 (53.8%) out of 13 patients with a history of preterm labor and 55 (42.3%) out of 130 patients with signs of irritation, itching and vaginal discharge were positive for *U. urealyticum* (Table 1).

As indicated in Table 1, the highest prevalence of *U. urealyticum*, according to cultural growth results, was among the patients aged 31 to 40 years; therefore, out of the 102 patients in this age group, 41 (40.2%) were positive.

Table 1. Distribution of *Ureaplasma urealyticum* Species in Different Age Groups

Age Group, y	Frequency (%)
< 30 (n = 48)	22 (9.5)
31 - 40 (n = 102)	41 (17.7)
> 40 (n = 82)	24 (10.3)
Total (n = 232)	87 (37.5)

Most of the patients had vaginitis. Among 130 patients, 55 (42.3%) were positive for *U. urealyticum* and out of 56 patients with a history of abortion, 23 (41.1%) were contaminated with *Ureaplasma* spp. (Table 2).

Table 2. Distribution of *Ureaplasma* spp. Infections Based on Clinical Symptoms

Infection	Frequency (%)
Infertility (n = 18)	7 (38.9)
Abortion (n = 56)	23 (41.1)
Vaginitis (n = 130)	55 (42.3)
Preterm labor (n = 13)	7 (53.8)
Total	87

## 5. Discussion

*Ureaplasma* species primarily colonize the mucus of the respiratory system and urogenital tract and have a close relationship with their hosts' epithelial cells. In some species of *Mycoplasma*, even the invasion of host cells is happen and the organism reside intracellularly. Sometimes the intracellular invasion is due to chronic infection and it enables the organism to evade from immune system (2).

Rapid laboratory detection of genital *Ureaplasma* species in pregnant females is very important, mainly because of the ability of the bacteria to colonize the endocervical lining and cause injury to the fetus. Epidemiologic data indicated that the presence of these bacteria in the genital tract is associated with incidence of infertility, miscarriage, sudden abortion, stillbirths and preterm births. Chorioamnionitis and postpartum endometritis in pregnant females and newborns (2, 11) due to bacterial infection is generally considered as the gold standard detection method of genital mycoplasmosis. The localization and linking to the host epithelial cells were important on colonization of *Ureaplasma* spp. and this importance led to a new method in this study.

There was no significant difference between age and accession, but a significant difference was observed in the mean age of the cases with signs of infertility (34.33%) and individuals without symptoms of infertility (37.57%) ( $P = 0.34$ ). Considering the age grouping of patients (Table 3), it was observed that the percentage of positive cases decreased significantly with increasing of age ( $P = 0.048$ )

**Table 3.** Relationship Between Age and the Percentage of Positive Cases

Age Group, y	Positive Cases (%)
< 30 (n = 48)	45.8
31 - 40 (n = 102)	40.2
> 40 (n = 82)	29.3

The results of the current study as well as those of other investigations, particularly those of Amirmozafari et al. (8) in Tehran, indicated that the prevalence of *Ureaplasma* spp. by culture was approximately 31.18%, which was lower than that of the current study (37.5%). In the current study, the method of cultivation, only reported the color change of the broth media. Amirmozafari et al. (11), conducted a similar study on 210 patients with clinical symptoms to compare PCR and culture results to detect genital *Mycoplasma* spp. It showed that out of 210 samples, 69 (32.9%) had positive culture or *Ureaplasma* spp. Furthermore, the highest prevalence of *U. urealyticum* was in the age group of 29 - 39

years, consistent with the results of the present study.

Suk-JuKimn et al. (12) conducted a similar study in South Korea on the endocervical swab samples and reported that out of 709 patients referred for screening urethritis, 22.1% were infected with *U. urealyticum*, which was also lower than the current study results (37.5%). The studies by Ekiel (13) and Baka (14) in which the endocervical sample swabs were cultured on commercial kits IST2 (the urea arginin broth) also indicated that the prevalence of *Ureaplasma* was 34.1% and 52.9% respectively. The difference could be due to cultural differences and the methods used.

Ahmadi et al. (15) performed a study to compare two methods, cultivation and PCR, in which the prevalence of *Ureaplasma* spp. with culture method was reported 32.7% that was similar to the results of the current study (37.5%). The study by Dilek Kilic et al. (16) in Turkey, culturing in IST2 media, reported the prevalence of *U. urealyticum* 48%.

The cause of variability of *Ureaplasma* spp. prevalence in different studies maybe due to the different diagnostic procedures and samples, different media, working on disparate populations and different geographical and cultural areas. The prevalence of *Ureaplasma* spp. infection was higher in people with multiple sexual partners; therefore, the high prevalence in the developed countries with sexual freedom was not unexpected.

Considering the fact that *U. urealyticum* species are one of the common factors of non-gonococcal urethritis (NGU) and urogenital diseases in females and males, the accurate diagnosis is essential.

The preparation of materials and equipment is necessary for laboratories to detect and treat urogenital diseases in females and plays an important role in accreditation and promotion of clinical microbiology.

### 5.1. Laboratory

The prevalence of *Ureaplasma* spp. was moderate in Qazvin, Iran. Considering the importance of these bacteria in pregnant females and the present risks for fetus and infant, more studies in this field are needed.

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